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INTRODUCTION

The investigation dealt with in the following paper concerns a cross between two species in the subgenus *Barkhausia* of the genus *Crepis*, both species having five pairs of chromosomes which exhibit, in somatic cells, size and shape differences in two of their five pairs. In addition, the members of the haploid sets show decided differences between one another.

C. foetida has four pairs similar in shape to four from *C. rubra*, but it lacks one corresponding to the fifth *rubra* chromosome, its place being taken by a duplication of another. Furthermore, one of the *rubra* satellited chromosomes frequently lacks its satellite in some strains, a situation existing in the two *rubra* parents represented in this study.

Numerous outstanding differences exist in the external morphology of the two species.

Little previous work has been done on this cross, beyond the determination of chromosome individuality, the occasional appearance of five bivalent chromosomes in the diaphase of F_1, and the supposed adherence of this hybrid to Navashin's scheme of "Amphiplastie" which has since been found to be erroneous in this case.

Navashin (1925) studied homology in ten *Crepis* species: one having three bivalents, six having four bivalents, and three having five bivalents. Therein he demonstrated the existence within his four-paired species of a B-chromosome not present in the three-paired species, *C. capillaris*, and also the existence in the five-paired species of an E-chromosome not represented in any species having less than five pairs. Both the species dealt with in the present paper were included in Navashin's study.

C. rubra was designated as having a haploid set A, B, C, D, and E, with C and D both satellited, the satellite attached to D being double and decidedly larger than the one attached to C. The smaller satellite itself, in all species except *C. rubra*, is a large well rounded body.

Difficulties in homologizing the chromosomes of these ten species begin when it is considered that the *rubra* chromosome C more nearly approaches in size and shape the D satellited chromosomes of *capillaris*

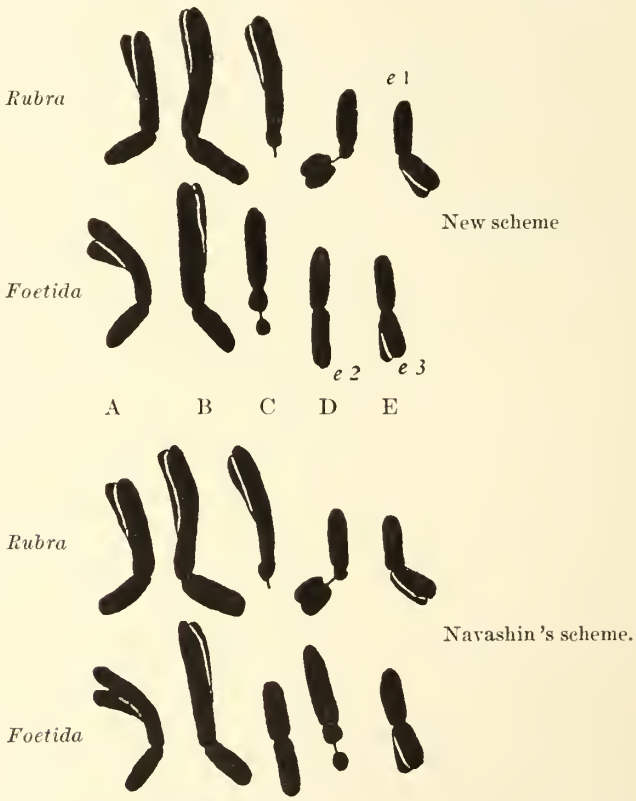

Fig. 1. Proposed schemes of chromosome homology in *C. rubra* and *C. foetida*.

and of all the four-paired species except *parviflora*, as well as the *foetida* chromosome he called D. Furthermore, *rubra* D in his scheme has no counterpart in any of the ten, when we consider the extra large and generally observable double nature of its satellite.

Other difficulties arose in describing the garniture of *C. foetida* subsp. *rhoedifolia*. All the subspecific forms of *Crepis foetida* have the same chromosome garniture. Here the E-chromosome is represented twice in the haploid set, whereas there is no second satellited

chromosome corresponding with *rubra* C. Consequently, Navashin designated the second E as *foetida* C, notwithstanding its obvious lack of relationship to *rubra* C.

The outstanding difficulty, then, lies in reconciling the fact that the F_1 hybrid frequently exhibits complete pairing of five homologues with the fact that the sets will not completely harmonize in Navashin's scheme. A picture of the situation may be obtained from figure 1.

The simplest solution seems to be to accept his designation of *rubra* as: A, B, C, D, and E; give the obviously homologous members of *foetida* the same letter, considering the *foetida* satellited one as the homologue of *rubra* C (which always has a satellite thread and sometimes a small satellite); and consider the extra *foetida* E^2 as homologous with *rubra* D, while the other is designated *foetida* E^3. As will be seen in the illustrated somatic garnitures, a comparison of *rubra* D with the E-chromosome shows sufficient resemblance to regard it as being a member of the heteromorphic pair which occasionally conjugates at the diaphase of F_1. The E-chromosomes have a median constriction, usually presenting the appearance of a perfect V. *Rubra* D has a short body ending in a subterminal constriction upon which there is a comparatively heavy thread, holding a double satellite. The total length of this chromosome usually agrees with that of an E-chromosome. This is especially true if we compare the length of the body of *rubra* D with that of one-half of an E-chromosome.

Evidence will be presented to show that the two now being called C both contain factors conditioning color of anther tubes and position of buds before anthesis.

In the following paper certain features of this hybrid and its derivatives will be discussed: (*a*) types obtained in the somatic garniture, (*b*) meiotic behavior, (*c*) the degrees of fertility and sterility, with probable causal agents, (*d*) morphology of the hybrids, and (*e*) deductions to be drawn from a combined cytological and genetic study.

COMPARISON OF THE PARENT SPECIES

The original cross giving rise to the majority of the hybrid derivatives under study, *C. rubra* (strain 1110) × *C. foetida rhoeadifolia* (strain 1539), was made in 1924 by C. W. Haney. Figure 2 shows the somatic garnitures of the two species. This cross will hereafter be referred to as Cross I. F_1 was backcrossed to both parents, with ease to *rubra,* but with difficulty to *foetida* in either direction. Conse-

quently a study of the selfed backcross derivatives must be largely confined to the *rubra* side.

No cytological study was made or notes taken upon the constitution of the F_1, the backcross generation, or the F_2 from this cross. Study was confined to the selfed backcross generation, all that was available when the present study was commenced. The author has thus far been able to obtain but one plant of the *rhoeadifolia* parent for root tip

Fig. 2. Somatic garnitures of *a, C. rubra; b, C. foetida typica; c, C. foetida rhoeadifolia.* × 3300.

cytological examination, and this died before reaching maturity. Buds from other plants of this parent strain, 1539, had been fixed previously, however, thus permitting an aceto-carmine study of meiosis in which irregularities were noted, as may be seen from figure 3.

The cytological studies following were made from material fixed and stained as shown below:

A. Root tips: fixed in Navashin 24 hours, stained in Haidenhein's haemotoxylin with standard schedule reported for all the *Crepis* investigations at Berkeley (Hollingshead and Babcock, 1930).

B. Buds: (*a*) Non-permanent: fixed in Carnoy one hour, and stained with aceto-carmine.

(*b*) Permanent: fixed in Carnoy 5 minutes; Navashin about 24 hours; stained in haemotoxylin, with schedule identical for that of root tips.

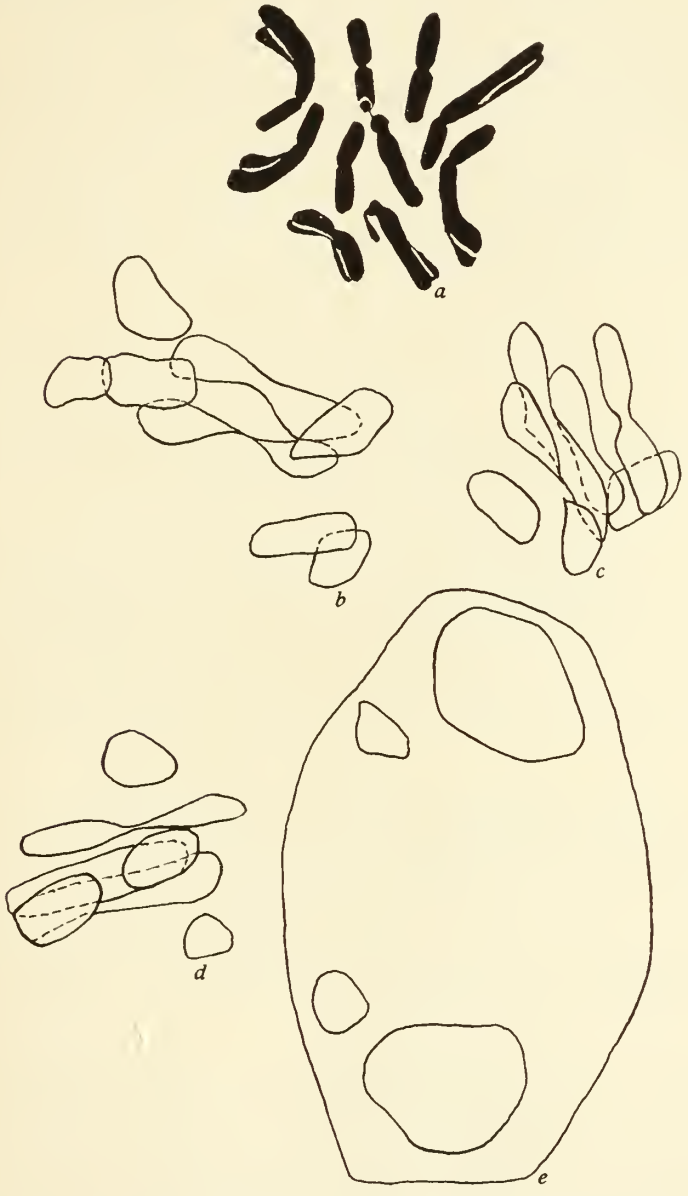

Fig. 3. Somatic and meiotic plates of *C. foetida rhoeadifolia,* strain 1539, used as parent in Cross I. *a,* somatic garniture; *b,* IM regular; *c, d,* showing non-conjunction of one pair; *e,* interphase, showing elimination of one pair.

From backcross studies it was hoped to identify the *foetida* characters accompanying such *foetida* chromosomes as could be distinguished. At first it was believed there were sufficient cytological differences between the two species to afford critical evidence on more than two pairs of chromosomes, because the members of the *rubra* set are consistently larger than the *foetida* homologues. It soon became evident, however, that no reliability could be placed on constancy of size differences in the hybrids, since three of the homologues, A, B, and E^1/E^3 afforded insufficient heteromorphism for utility in this connection; and furthermore, that, despite numerous external morphological differences, they are mainly of a quantitative nature and relatively few are suitable for correlation studies.

C. foetida rhoeadifolia, strain 1539, despite its greater sterility, was chosen as parent in preference to *C. foetida typica* because it offered several character pair contrasts with *rubra* not available in the latter, notably: (*a*) erect buds, (*b*) a set of long coarse eglandulose hairs on the involucral bracts, (*c*) a different shape of the outer bract itself, and (*d*) a taller plant, frequently exceeding 100 cm., whereas *typica* is not much taller than *rubra.* Except for certain achene differences, a wider distribution of anthocyanin over the stem, branches, and leaves, the remaining characters are those of *foetida typica.*

The *rubra* parent was of the varietal form *alba,* in which the ligules are white, the anther tubes purple or lavender, and the pollen grains white.

In addition to Cross I, a second cross has been utilized, in which *foetida typica* was crossed with a white-flowered strain of *rubra* practically identical with the one occurring in Cross I.

Plate 9, figure *a,* shows four plants, from left to right: *foetida typica,* F_1 (*foetida typica* × *rubra*), *rubra,* and a derivative of the *foetida rhoeadifolia* parent. The branching habit of all four types is well illustrated and *rhoeadifolia* is seen to be quite luxuriant when mature.

Cross II was performed by M. Navashin in Moscow, and the F_1 was raised in Berkeley. Only one plant was selfed, giving rise to the most interesting of the derivatives thus far obtained.

Foetida typica is highly fertile, but *rhoeadifolia* is much less so. A glance at figure 3, *a, b, c,* and *d* indicates that the meiotic irregularities occurring in plants of the strain of *rhoeadifolia* used for parent in Cross I may be partly associated with a difference in the

size of the satellites found in every somatic plate, examined from the only plant of strain 1539 available. This, together with some lack of genetic homology, may account for the lower fertility of *rhoeadifolia* compared with *foetida typica.* A single pair of chromosomes shows non-conjunction in meiosis and the somatic garniture indicates that the satellites on the two C-chromosomes are never the same size. Figure 3*e* is drawn to the same scale as the other meiotic figures and it is evident that the excluded chromatin material adjoining the two interphase nuclei exactly correspond in size to the non-conjugating chromosomes of 3*d*. Therefore, irregularities in one pair may be causing all the sterility. Pollen grain counts are not yet available in this strain.

CROSS I

In summarized tabular form, the character contrasts afforded by Cross I (*C. rubra,* 1110 × *C. foetida rhoeadifolia,* 1539) are:

Rubra	Foetida
Stems	
Stems numerous, almost glabrous, scapose	Stems solitary, branched, leafy whole length, pubescent
No anthocyanin (rarely any)	Anthocyanin widespread
Leaves	
Rosette dark green, erect, almost glabrous	Rosette gray green, flat, pubescent
Buds	
Nodding before anthesis	Erect before anthesis
Outer bracts glabrous	Outer bracts with coarse eglandulose hairs
Outer bracts ovate lanceolate, margin scarious	Outer bracts broadly lanceolate, margin not scarious
Flowers	
Ligules white, both sides	Ligules strontian yellow, with median red stripe on outer side
Anther tubes purple	Anther tubes yellow
Style branches white	Style branches greenish brown or yellow
Pollen grains white	Pollen grains yellow
Open all day	Open forenoon only
Achenes purplish brown, inner ones 16–20 mm.	Achenes light brown, inner ones 12–16 mm.
Diameter open head 47 mm.	Diameter open head 29–38 mm.
Miscellaneous	
Height *ca.* 26 cm.	Height *ca.* 100 cm.
Odor slightly *foetida*	Strongly *foetida*

The present study was begun with achenes of (*a*) the backcross to *rubra* selfed, in which thirty plants reached maturity, from over one hundred germinated (one, a triploid, and the root tips of two others were lost); and (*b*) the backcross of *foetida* selfed, of which only seven plants germinated or reached maturity. The thirty plants in (*a*) were derived from six plants of the backcross generation (see table 1). An attempt is being made to duplicate as many as possible of the earlier stages of the investigation.

In the seven plants from (*b*), the morphology was predominately *foetida,* and the somatic metaphase plates were apparently all of *foetida*-like appearance, with two exceptions to be discussed later. However, so small a generation provides such inadequate means for correlation of cytological and morphological evidence as to be practically useless.

THE BACKCROSS TO *RUBRA*

With the twenty-eight plants from the backcross to *rubra,* however, the situation is different. Sufficient material is provided to permit rather definite conclusions regarding: (*a*) distribution of chromosomes from the two original parents, as well as (*b*) a correlation between such *foetida* chromosomes and characters as are distinguishable.

As previously noted, but two of the five homologues are sufficiently heteromorphic for service in this study, the pairs C_rC_f and DE^2. For each pair, then, the backcross population would show a distribution of .5 RR + .5 RF, which on selfing would give a population distributed in the proportions, .625 RR + .25 RF + .125 FF. In order to determine the distribution for any given number of pairs of distinguishable chromosomes, this expression may be raised to the corresponding power. In this instance the formula would be represented as $(.625\,DD + .25\,DE^2 + .125\,E^2E^2)\ (.625\,C_rC_r + .25\,C_rC_f + .125\,C_fC_f)$.

Multiplying the two members of this formula will give us types which are readily distinguished in somatic metaphase garnitures, illustrations of which for the two parents may be seen in figure 2, and for F_1 in figure 4. The types existing among the hybrid derivatives may be roughly divided into three main groups corresponding to the two parents and F_1. The distinction between such types depends solely on the distribution of the pair D/E^2, and for convenience in subsequent reference such types may be designated by the suffix oid; e.g., (*a*) rubroid, (*b*) foetoid, and (*c*) funoid. Each of these three

groups may in turn be subdivided into three groups according to the distribution of the pair C_r/C_f. Among these nine types those in which the homologues are heteromorphic may be further distinguished as pseudo-rubroid rf, or ff, pseudo-foetoid rr, or rf, and pseudo-funoid rr, or ff.

Upon this basis comparisons may be made between the number of such somatic types actually observed and the numbers calculated by solution of the formula now under consideration.

Fig. 4. Somatic and IM plates of an F_1 hybrid between *C. rubra* and *C. foetida typica*. *a*, somatic garniture; *b*, IM, showing five bivalents (aceto-carmine preparation).

TABLE 1

Comparison of Observed with Expected Somatic Garniture Types

Somatic type	Observed	Per cent	Calculated
Rubroid C_rC_rDD	11	39.3	39.1
Pseudo-rubroid C_rC_fDD	5	17.85	15.6
Pseudo-rubroid C_fC_f	5	17.85	7.8
Funoid $C_rC_fDE^2$ (C_rC_r, C_fC_f)	3	10.714	25.0
Foetoid $C_fC_fE^2E^2$	2	7.143	1.6
Pseudo-foetoid $C_rC_fE^2E^2(C_rC_r)$	2	7.143	10.9

The discrepancies thus obtained are probably due to several causes, chiefly that (*a*) of the six cultures representing this selfed backcross generation, three have but one plant each and two are fairly well represented, and (*b*) the six parent plants probably do not represent a random sample of the backcross generation (see table 2).

TABLE 2

CORRELATION OF FOETIDA CHARACTERS WITH FOETIDA CHROMOSOMES IN CROSS I

Plant No.	Foetida characters	Somatic type	Per cent good pollen
28.001–1	Red stripe, purple tips	p. rubroid C_rC_f	
3	Red stripe, anthocyan	rubroid C_rC_r	82.9
4	Yellow anther tubes, erect buds, anthocyan	p. rubroid C_fC_f	
9	Yellow anther tubes, erect buds	p. rubroid C_fC_f	
10	Yellow anther tubes, erect buds	p. rubroid C_fC_f	70.5
11	Red stripe, anthocyan, long life	rubroid C_rC_r	
12	Central stem, red stripe, yellow stigmas, anthocyan, hairy leaves, long life	rubroid C_rC_r (4n sector)	
13	Central stem, red stripe, anthocyan, purple tips	p. rubroid C_rC_f	
14	Erect buds, red stripe, yellow anther tubes, long life	p. rubroid C_fC_f	
16	Anthocyan, hairy leaves	rubroid C_rC_r	
18	Erect buds, yellow tube, red stripe, long life	p. rubroid C_fC_f	
19	Anthocyan	rubroid C_rC_r	
20	Red stripe, stigmas yellow, anthocyan	rubroid C_rC_r	
28X40.1	Yellow pollen, anthocyan, pubescence, yellow anther tube, yellow ligules	triploid (2n=foetoid $C_fC_fE^2E^2$) (n = rubroid C_rD)	
2	Thirteen *foetida* characters, nodding buds	p. foetoid $C_rC_fE^2E^2$	24.6
3	Ten *foetida* characters, nodding buds	funoid $C_rC_fD\ E^2$	34.2
4	Eight *foetida* characters, nodding buds	p. foetoid $C_rC_fE^2E^2$	
28X42.1	Anthocyan, red stripe	(lost roots)	
5	Anthocyan	rubroid C_rC_r	
17	Anthocyan	rubroid C_rC_r	
18	Anthocyan, red stripe	rubroid C_rC_r	
21	Anthocyan	rubroid C_rC_r	97.9
22	Anthocyan, colored tips	p. rubroid C_rC_f	
23	Anthocyan, colored tips only, narrow head	p. rubroid C_rC_f	90.7
24	Anthocyan	rubroid C_rC_r	86.3
25	Anthocyan, colored tips	p. rubroid C_rC_f	94.1
27	Anthocyan	(lost roots)	97.7
29Z 6	Fourteen *foetida* characters, nodding buds	foetoid $C_fC_fE^2E^2$	57.3
28X43.1	Colored tips, nodding buds	funoid $C_rC_fD\ E^2$	
28X44.1	Yellow anther tube, erect buds, yellow ligules, pubescence	foetoid $C_fC_fE^2E^2$	

The relevant data from the twenty-eight plants constituting this generation are included in table 2, where the plants comprising the six cultures represented are listed with their *foetida* characters, *foetida* chromosomes, and the percentage of good pollen grains.

The two cultures in which there is a fair representation of the selfed generations are 28.001 and 28X42. Their examination may provide some information concerning the manner of distribution of the heteromorphic pairs under study.

It will be seen from the above table that there are eleven rubroid (C_rC_r) plants, five pseudo-rubroid (C_rC_f), and five pseudo-rubroid (C_fC_f) plants, one of which was doubtful.

The agreement between the observed and calculated distribution is:

	C_rC_r	C_rC_f	C_fC_f
Calculated	13.125	5.25	2.625
Observed	11	5	5
Deviation		± .25	

The probable error ($0.6745 \sqrt{\text{p. q. n.}}$) for such a population is 1.35 and the probability of such a deviation being due to chance is about 90 per cent on a basis of three homomorphic to one heteromorphic.

Such data for the pair DE^2 will be obtained from an inspection of all twenty-eight plants, and when analyzed in a similar manner the distribution is:

	D D	D E^2	E^2E^2
Calculated	17.5	7.0	3.5
Observed	21	3.0	4.0
Deviation		± 4.0	

In this case the probable error is 1.55 and the probability is only 8.9 in 100; but, as previously pointed out, the disparity in numbers between the six cultures is not expected to furnish critical data.

On plate 10 there are three photographs, showing growth habits of plants representing cultures (*a*) 28X42, (*b*) 29Z6, and (*c*) 28X40, together with *rubra* and a *rhoeadifolia* derivative quite similar to the parent strain. Notice in *a* the almost perfect resemblance of 28X42 (29Z8) to *rubra* (R 1110), and the highly variable expression of the

three plants representing 28X42 in photograph (*c*). One of them is almost identical with *rhoeadifolia*, while the other two obviously show intermediacy.

Therefore, such critical evidence as exists points to the random (*a*) segregation of chromosomes, (*b*) formation of gametes, and (*c*) assortment of phenotypes.

CORRELATION OF CHROMOSOMES WITH CHARACTERS

A closer inspection of the population 28.001 shows that among the six so-called rubroid (C_rC_rDD) plants there is quite a range of *foetida* characters in evidence. Plant 19 exhibits but one *foetida* character, while the others show more, and plant 12 shows six of them. Yet in the cytological examination of these six plants it was impossible to distinguish any *foetida* chromosome. This means that the isomorphic pairs, A, B, and E, comprise some *foetida* chromosomes, or there has been some interspecific crossing over, or both.

With regard to the seven pseudo-ruboid plants, however, two of which have C_rC_f and five C_fC_f, it will be noticed that those having C_rC_f have the purple on the anther tubes restricted to the tips, whereas those having C_fC_f have, among their *foetida* characters, yellow anther tubes, as well as erect buds. The C_rC_r plants show entirely purple anther tubes and nodding buds. Plant 28X44.1 ($C_fC_fE^2E^2$) agrees with the five C_fC_f plants of 28.001 in having yellow anther tubes and erect buds.

The ten plants constituting culture 28X42 likewise show assortment of the C_f chromosome, three plants being classified as C_rC_fDD and the remainder as C_rC_rDD. Those with C_rC_fDD have restricted color on the anther tubes, whereas those having C_rC_rDD are solid colored. All plants have nodding buds.

Photographs of three plants of 28X42, figure *a*, plate 10, illustrate the predominance of *rubra* characters, compared with the *rubra* parent shown alongside. It will be noticed from table 2 that very few other *foetida* characters occur in any of the plants, and the presence of anthocyanin, common to all, is doubtless contributed from both species, although it is seldom seen in pure *rubra* plants.

However, the fact that all nineteen plants of cultures 28.001 and 28X42 have two *rubra* D-chromosomes, may raise some doubts concerning the proposal for homologizing C_fC_r instead of accepting

Navashin's homology of DC_f. Such doubts are dispelled by the fact that plant 28X44.1 ($C_fC_fE^2E^2$) and the three diploid plants of F_2 in Cross II, see table 4, all confirm the above evidence and in addition all lack any D-chromosomes. This proves that C_r alone contains the factor which would disturb yellow anther tube pigmentation, causing purple anther tips when together with C_f. Even though the gross morphology of these plants suggests *foetida*, all of them agree in possessing this one *rubra* character, as well as the C_r and not the D.

Furthermore, the three triploids of Z14 all have the C_r and colored anther tube tips, whereas Z13.2 (also $3n$) was classed as having entirely purple anthers tubes and since its extra set was C_rD, this is to be expected. The question of bud position does not enter in Cross II because the strain of *foetida typica* which was used in this cross has only nodding buds.

Returning again to Cross I, as shown in table 2, there are four plants in the population 28X40, the gross morphology of which, as well as the somatic garnitures, is predominately *foetida*. 28X40.1 is a triploid, and the somatic plate discloses a situation which can only be interpreted on the assumption that the diploid gamete was foetoid ($C_fC_fE^2E^2$) and the haploid was rubroid (C_rD). But for the non-reduction of one of the gametes this plant would have been a funoid ($C_rC_fDE^2$) and has been so classified in table 2. The plant possessed yellow anthers and nodding buds. The remaining three plants of this culture all agree in having C_rC_f. Morphologically they have nodding buds as expected, but also yellow anther tubes, not expected from the foregoing discussion.

Of the three remaining plants of this backcross selfed generation, 28X44.1 is C_fC_f, and has yellow anther tubes as well as erect buds. 29Z6 being also C_fC_f agrees in having yellow anther tubes but disagrees in having nodding buds, to be seen from photograph *b*, plate 10. Plant 28X43 is C_rC_f and shows nodding buds and colored tips, as expected for a funoid plant.

According to the facts, the hypothesis may be advanced: that C_rC_r gives purple anther tubes and nodding buds, C_rC_f gives purple tipped yellow anther tubes and nodding buds, and that C_fC_f gives entirely yellow anther tubes and erect buds, barring the incidence of interspecific crossing over. We then have perfect agreement in thirteen plants from five cultures, but disagreement in four cases, representing but two cultures, and these cultures are not well represented numerically.

The discrepancy in the matter of yellow anther tubes for C_rC_f in the three plants of 28X40 might easily have resulted from an error in classification, but there is an alternative explanation in view of the fact that *Crepis* workers recognize that in some *rubra* strains there is variability in the intensity and extent of the purple pigmentation on the anther tube.

In the case of bud position the discrepancy is still more readily disposed of, since erect buds are dominant in intraspecific crosses in *C. foetida*, although recessive in all interspecific crosses, and the parental strain 1539 is known to be heterozygous for the character. Doubtless the one plant used as parent in Cross I was heterozygous. In all cases nodding buds are dominant over erect buds in derivatives.

The possibility that these exceptions are due to interspecific crossing over is to be further investigated with a larger F_2. If only the three phenotypic classes found this year are then obtained there will be indication of no crossing over, but if the six possible phenotypes are obtained, then the linkage value can be computed from the frequency of the yellow-anther-tube erect-bud class, since it will be the double recessive class. The situation may be represented as below:

E, nodding buds — e, erect buds
P, purple tubes — p, yellow tubes

F_1 $\frac{EP}{ep}$

Expected types in F_2:

EP, nodding buds, purple tubes;
EPp, nodding buds, purple tipped tubes;
Ep, nodding buds, yellow tubes;
eP, erect buds, purple tubes;
ePp, erect buds, purple tipped tubes;
ep, erect buds, yellow tubes.

Regardless of any question as to whether or not interspecific crossing over has or will occur, the situation with respect to a correlation between the character pairs and the chromosome pairs may be summarized as follows: agreements with the hypothesis are consistent in the two cultures having the most significance, i.e., 28.001 and 28X42, whereas the discrepancies are fairly easily disposed of. Therefore, chromosome C, whether C_f or C_r, may be considered to contain the factors conditioning coloring of the anther tubes and position of the buds before anthesis. Furthermore, the fact that the three diploid members of the F_2 population of Cross II agree with the hypothesis, and at the same time lack the *rubra* chromosome D, proves that D_r cannot be homologous with C_f, as Navashin tentatively assumed in 1925.

THE F_1 GENERATION

In an attempt to duplicate some of the earlier steps of this cross, a strain of *foetida typica* was crossed with a white-flowered strain of *rubra,* both similar to the parents employed by Navashin in Cross II. By so doing it was hoped to avoid the sterility introduced into Cross I by the use of *foetida rhoeadifolia.* One F_1 individual was secured from this cross. A photograph of it, showing the intermediate expression of habit and size between the adjacent parents is illustrated in plate 9. Its somatic garniture is illustrated in figure 4*a*, showing the convenient distinctions between the two parents set up by the two heteromorphic pairs D/E^2 and C_r/C_f.

Owing to a limited number of flower-heads, and the desirability of utilizing as many as possible in the production of backcrosses and F_2 individuals, only a few buds were fixed for a study of meiosis. These were stained for temporary aceto-carmine examination. In preparation of *Compositae* mounts by this method such a large number of PMC's are eliminated in removal of sporophytic tissue that usually relatively few division figures are retained. Furthermore, the chromosomes are more swollen and larger than those of material fixed for permanent mounting.

The one slide obtained confirmed the fact that five bivalents occur in F_1. See figure 4*b*. Nothing was then learned of the frequency of occurrence of complete pairing, as studied by Babcock and J. Clausen (1928) in three hybrids between four-paired species, but deductions based on the percentage of good pollen grains place the figure around 2.7 per cent, as shown in table 4.

It would be highly desirable to know something of the frequency of occurrence of unreduced gametes in F_1. An estimate may be made indirectly from F_2 data. From the nine plants of this population (see table 4) the eighteen parental gametes consisted of seven diploid and eleven haploid gametes, or approximately 39 per cent unreduced, and 61 per cent reduced.

MEIOSIS IN A FUNOID PLANT

Since the chief interest in Cross I is attached to a study of external morphology and chromosome individuality, few of the hybrid derivatives needed to be studied for the reduction division. The fact that

the four plants from 28X40 showed more than the usual number of *foetida* characters, however, suggested the advisability of such study here.

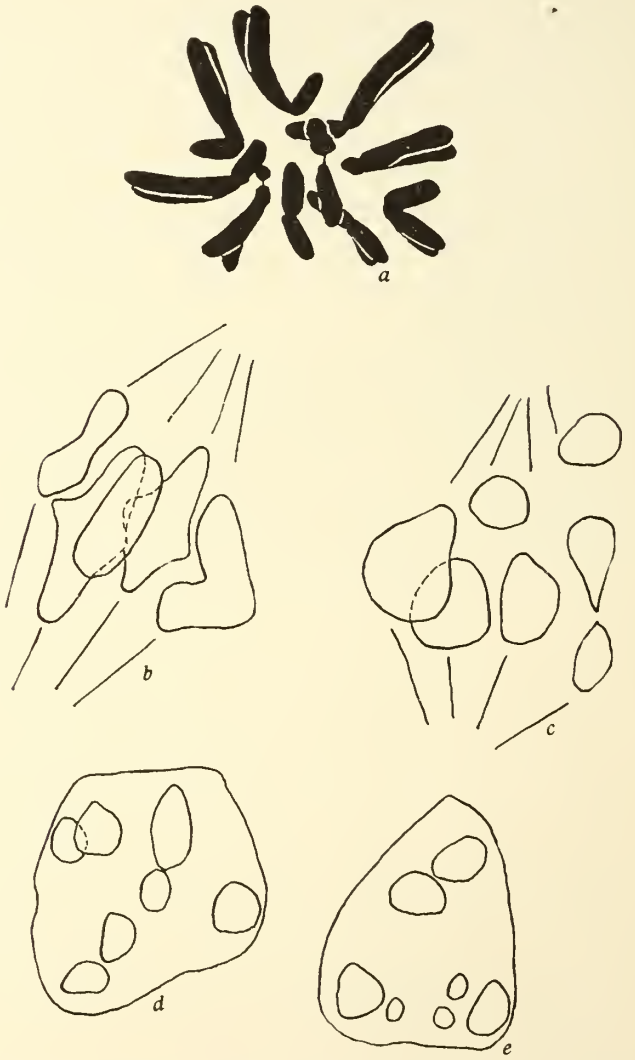

Fig. 5. Drawings from a funoid derivative ($C_rC_fDE^2$). *a*, somatic garniture; *b*, IM, showing five bivalents; *c*, non-conjunction in one pair; *d*, *e*, tetrad stages.

Figure 5*a*, an illustration of what is meant in the preceding discussion by a funoid garniture ($C_rC_fDE^2$), admirably shows a plate apparently identical with the garniture of a true F_1 plate as illustrated in figure 4*a*. In the two late metaphases, shown in figure 5*b*

and *c*, are shown situations that might be expected from our previous knowledge of occasional complete pairing in F_1, together with irregularities, in this case non-conjunction of two univalents. From figure 5*d* and *e*, however, the situation is seen to be still more complex than would be expected from a single pair of non-conjugating chromosomes.

In table 2 it is seen that the *percentage of good pollen* for this plant is 34.2 per cent. In table 4, as discussed above, it is shown that for F_1 plants the *percentage of good pollen* is only 2.7 per cent. Now, obviously in the latter case we have the maximum amount of heterogeniety to be expected in hybrid derivatives; therefore, with eight times as many well stained pollen grains, we may conclude that 28X40.3 contains true homology in some of the three more or less isomorphic pairs of chromosomes (i.e., A's, B's and E's).

From the evidence thus far considered in the backcross to *rubra* it is clear that study of a larger backcross generation, as well as of a larger selfed backcross generation, will prove invaluable in elucidating some perplexing problems of interspecific hybridization, e.g., character-chromosome correlations, method of chromosome distribution, and viability of gamete types.

THE BACKCROSS TO *FOETIDA*

Selfed backcrosses to the *foetida* parent proved so sterile that only seven achenes were viable. Had the resultant population been of sufficient size it would have been desirable to have ordered the investigations along the line of the backcross to *rubra*, with the exception of attempted correlation of *rubra* chromosomes with *rubra* characters. Inability to conduct this phase of the problem further emphasizes the inconvenience of having used *rhoeadifolia* instead of *foetida typica*, because of the sterility ensuing from abnormal reduction.

A glance at table 3 shows that all the plants of the backcross generation to *foetida*, with the exception of 28Z9.2 and Z9.3, exhibited a foetoid garniture and resembled *foetida* in morphology. These exceptions have nodding buds and are both C_rC_f, again according to expectation (see pl. 9, fig. *b*).

Notes taken during morphological examination mention only yellow anther tubes in all cases, but it is entirely possible that colored tips in the pseudo-foetoid plants were present and overlooked. It will be noticed that all C_fC_f plants have erect buds. The data gathered in 1928 were not as complete as those gathered later.

The predominant type of garniture represented in this selfed backcross is foetoid, which is to be expected from the predominance of rubroid types in the reciprocal backcross.

The three plants growing in 1929 are shown in plate 9, figure *b*, where the *rhoeadifolia* habit of growth is evident.

TABLE 3

RUBRA CHARACTERS AMONG PLANTS OF THE ONE SELFED FOETIDA BACKCROSS

Plant	Rubra characters	Somatic type	Per cent good pollen
28X45.1	——	foetoid C_fC_f	
28Z 9.1	No red stripe, erect buds	foetoid C_fC_f	
2	Nodding buds	p. foetoid C_rC_f	49.3
3	Nodding buds	p. foetoid C_rC_f	55.3
29Z 9.1	Open all day, medium height, erect	foetoid C_fC_f	93.8
2	Open all day, short stature, broad head, erect	foetoid C_fC_f	46.2
3	Open all day, broad head, erect	foetoid C_fC_f	60.8

CROSS II

There were about six F_1 achenes of Cross II brought to Berkeley from Moscow by Dr. Navashin. One of these was transported to the Genetics Division garden at Palo Alto. Several heads of this plant were bagged and at the proper time all normal looking achenes were gathered and labeled. The plant had been isolated from other *Crepis* species, obviating the chances of crossing in the open pollinated heads. In the spring of 1929 the seed from this selfed plant was sown at Berkeley, and all plants that germinated reached maturity; three from the open heads and six from the bagged heads. Examination of root tip material of these nine plants revealed the situation shown in table 4.

There are three diploids, all of the pseudo-foetoid (C_rC_f) type, five triploids with constitution denoting an unreduced F_1 gamete plus: one rubroid, two foetoids, and two pseudo-foetoids, respectively.

Photographs of these nine plants are shown on plate 11 where an excellent idea of the gross morphology of each plant may be formed. Notice especially the resemblance of the amphidiploid to the F_1 branching habit illustrated in plate 9, figure 1; also how the triploids derive their gross morphology from the parent represented by the assumed extra set. For instance 13.2 is shorter in stature and has much broader heads than 13.3, the former having a rubroid set

extra, the latter a foetoid set extra. All six plants of 29Z14 appear to resemble *foetida* uniformly, yet three of them are $2n$, and three are $3n$. All six, however, have purple tips on their anther tubes, a *rubra* character. All six have the C_r and only three have D, thus the D appears to have no effect in conditioning the color of anther tubes, as previously noted.

TABLE 4

F_2 Generation of Cross II

Plant Ns	Rubra characters	Somatic type	Per cent good pollen
29 Z13.1	Intermediate	amphidiploid	34.5
2	Characters mostly *rubra*, purple tips	3n, ex. set C_rD	20.0
3	Characters mostly *foetida*, yellow anther tubes	3n, ex. set C_fE^2	36.5
29X14.1	Short life, white ligules, purple tips, outer bracts *rubra* shaped	2n, p. foetoid C_rC_f .	20.2
2	Characters mainly *foetida*	3n, ex. set C_fE^2	29.7
3	Characters mainly *foetida*	3n, ex. set C_fE^2	17.9
4	Characters mainly *foetida*	3n, ex. set foetoid C_fE^2	9.0
5	Purple tips, *rubra* outer bracts, bracts glabrous	2n, p. foetoid C_rC_f	8.3
6	Purple tips	2n, p. foetoid C_rC_f	38.2
	Rubra strain 1110		96.6
	Foetida typica (strain similar to that of Cross II)		95.4
	F_1 (*foetida typica* x *rubra alba*)		2.7

From these data it is difficult to say in the triploids whether it is the megaspore or microspore that has been unreduced; but from the occurrence of unreduced gametes in 39 per cent of the cases, and the chance meeting of two of them to produce an amphidiploid, there can be little question that both gametes on the same plant are affected. Figure 6*a* excellently depicts the complete diploid complements of both parents in the amphidiploid.

From table 4 it is seen that this plant has only 34.5 per cent good pollen, almost identical with the percentage found by Hollingshead (1930) in her *capillaris-tectorum* amphidiploid. One immediately suspects from this that irregularities of the reduction division are responsible. Accordingly an investigation was begun into its meiotic behavior.

Fig. 6. Preparations from an amphidiploid. *a*, somatic garniture; *b*, *c*, diaphases showing five and seven units; *d*, *e*, *f*, IM showing five, six, and seven units.

Paraffine sectioned buds of *Compositae* material are especially favorable for such a study and disclose a wide range of conditions on adjacent concentric circles of florets. The outer row may have pollen mother cells in the tetrad stage, the second row, or only part of it, may have second division figures, while the remainder of that row, and part of the third are in first metaphase or earlier. At the same time in the center of the bud some of the tissue may not yet have reached the gametophyte stage. Consequently when first division figures are present practically all information desired is available, if the fixation is good. This situation is quite unlike that found when aceto-carmine mounts are used, for in such a case most of the PMC's would remain within the anther tubes.

From a study of figures 6 and 7 it is evident that quadrivalents are frequent. In the plates illustrated it will be seen that cell complements range from five to thirteen units at the first metaphase. Such variability in conjugation would naturally result in irregularities at the end of the second division, shown in figure 7*e*.

When one finds five bivalents in F_1 it is not surprising that five quadrivalents should occasionally be found in a $4n$ individual, and the fact that intergrading types of conjugation occur, with consequent elimination of microcytes and micronuclei, would certainly account for the major portion of the 65.5 per cent non-staining pollen grains obtained in aceto-carmine determinations. However, a sufficient number of viable gametes are produced to insure a fair degree of fertility upon selfing. From twenty-two heads fifty-five achenes were gathered. This shows a higher degree of fertility than in the best diploid, 29Z14.6, which produced fifty achenes from about forty-two heads.

Evidently when the phenomenon of amphidiploidy occurs in more or less closely related species the occasional formation of quadrivalents (in this instance, at least), and their subsequent random assortment, so upsets meiotic division that irregularities in the production of gametes operate to the disadvantage of such hybrids, as contrasted with hybrids between more distantly related species in which no pairing at all occurs, e.g., *Raphanus-Brassica, Nicotiana bigelovii-N. glutinosa.*

Further studies of Cross II, especially the progeny of the amphidiploid and the two fertile diploids, will be reported in a subsequent paper.

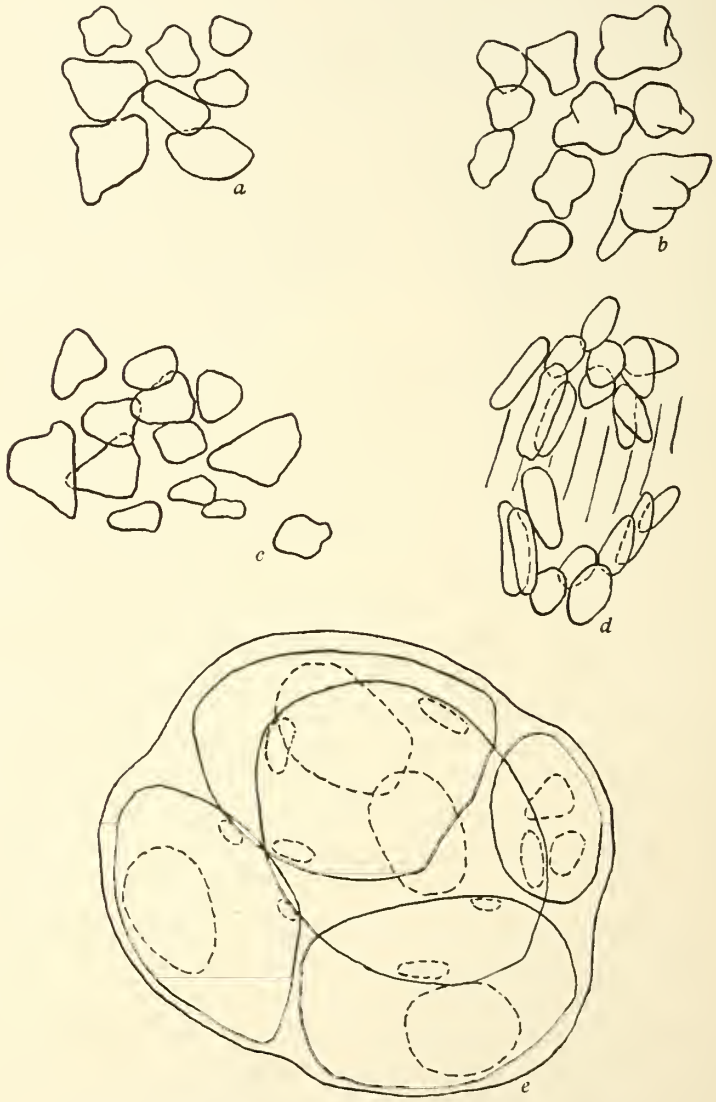

Fig. 7. From the same amphidiploid as in figure 6. *a*, *b*, *c*, IM showing eight ten, and thirteen units; *d*, IA, showing nine to eleven univalents; *e*, tetrad stage, with microcytes.

STERILITY AND VIABILITY

Use of the *percentage of good pollen* as an indication of fertility was not attempted generally until 1929. Therefore, populations grown in 1928 show few data on this important point. Glancing over the column per cent of good pollen in tables 2, 3, and 4, it is seen that if cultures are considered as units a fairly consistent range of fluctuation occurs. In culture 28X42 the percentage of good pollen in the five members so tested is uniform and high; in culture 28X40, the two members tested are in agreement; in culture 29Z12, all the members tested are uniform and low. The seven members representing the backcross to *foetida* likewise show some degree of uniformity, with one exception. In view of this fact it may be considered that the two determinations of 70.5 per cent and 82.9 per cent good pollen in cultures 28.001, would roughly represent the degree to be found there had more material been studied.

Such being the case it is seen that if this character be considered a measure of fertility, then those combinations representing the maximum degree of heterozygosis (as in F_1, or populations where a large number of *foetida* characters are encountered in the backcross to *rubra*), will exhibit the lowest percentages of good pollen, as tables 2, 3, and 4 show.

On the other hand, environmental factors exert a considerable rôle in sterility, as seen by the fact that no seed at all was set from culture 28.001 in September, where the determinations of good pollen were high; whereas in late spring and summer, viable seed is obtained from F_1 plants, with but 2.7 per cent good pollen.

From table 4 it will be seen that the last two plants in culture 29Z14, both diploids, exhibit very low percentages of good pollen. Yet both these plants were fertile, producing twenty and fifty achenes respectively, a high proportion of which have germinated. The only other plant in this population to set any achenes was the triploid Z13.3, which produced only two.

In a study of meiosis in some *Crepis* species and hybrids, Babcock and J. Clausen (1929) show that the closer the phylogenetic relationship between the species the greater the degree of fertility and the higher the percentage of good pollen grains in the hybrids. Thus, in more distantly related species, exen though their respective chromo-

somes may agree in size and shape, their genetic identity has been so altered in the course of time that homology of chromosomes is at a minimum.

Hollingshead (1930) shows that even in triploids of *C. capillaris* × *C. tectorum*, where the former is represented twice in the somatic garniture, the percentage of good pollen grains is much higher than in F_1, and undoubtedly the degree of homology in such a triploid is higher than in an F_1 diploid.

In view of these facts it may be said that percentage of good pollen grains is an indication of homology of chromosomes, but affords little idea of the proportion of viable seed to be expected.

ACKNOWLEDGMENT

Grateful acknowledgment is due Professors E. B. Babcock and R. E. Clausen for suggestions and criticisms made during this study.

SUMMARY AND CONCLUSION

There are two subspecific forms of *C. foetida* used in this study, *rhoeadifolia* and *typica*, and but one of *C. rubra*. All have five pairs of chromosomes, of which three pairs are obviously homologous in somatic metaphase plates, whereas the homology of the two remaining pairs, which are known to conjugate in F_1, has been inferred from the cytological and genetic evidence.

Of the sixteen morphological character pairs used in genetic analysis, two have been assigned to the interspecific homologues designated C_rC_f.

The character pairs, purple-yellow anther tubes and nodding-erect buds before anthesis, the former of each pair from *rubra*, the latter from *foetida rhoeadifolia*, are definitely correlated with the chromosome pair C_rC_f, as used in homologizing the haploid sets of these two species.

C_rC_r, purple anther tubes, nodding buds;
C_rC_f, purple tipped yellow anther tubes, nodding buds;
C_fC_f, yellow anther tubes, erect buds.

C. foetida rhoeadifolia is much less fertile than *C. foetida typica*. Somatic garnitures of the strain 1539 of the former subspecies exhibit

a difference in the shape of the satellites attached to the chromosomes designated C. Cytological preparations of meiotic divisions indicate failure of one pair of chromosomes to conjugate in metaphases, with subsequent elimination of one pair at the conclusion of the first division. It is suggested that the irregular meiotic pair may be identical with the non-uniform somatic pair, and that some of the lessened fertility of *rhoeadifolia* in nature is due to partial loss of genetic homology in the pair.

The fact that no aneuploid individuals have thus far issued from derivatives of the cross *rubra* × *foetida* indicates that gametes lacking entire sets of five or ten chromosomes are mostly non-viable. So far as ascertainable, no other chromosome combinations are non-viable.

Cultures issuing from single self-pollinations agree rather closely in (*a*) percentage of good pollen gains, and (*b*) the similarity of somatic garniture types, but not in the degree of fertility; therefore, percentage of good pollen grains is useful as an index of chromosome homology, but not degree of fertility.

In selfing the backcross of F_1 to *rubra* there was obtained among the better, numerically represented cultures, a sufficient range in chromosome assortment from the two species, so that upon the basis of random distribution of the two heteromorphic pairs C_rC_f and DE^2, a close approximation was obtained of the expected with the observed assortment.

Meiosis of plants showing maximum degrees of heterozygosis, i.e., true F_1 and funoid types ($C_rC_fDE^2$), exhibit instances of complete pairing. An amphidiploid plant of this cross exhibited instances of complete quadrivalence. The population from which the amphidiploid arose indicated that unreduced gametes existed in both sexes, and that among the nine ensuing F_2 plants there were one amphidiploid, five triploids, and three diploids; therefore a ratio of eleven reduced to seven unreduced gametes.

Although the two species *rubra* and *foetida typica* show a considerable number of distinct morphological differences, there is sufficiently close relationship in the amphidiploid that homologous chromosomes from the two species tend to form quadrivalents in varying frequency during meiosis, and the ensuing irregularities at reduction cause a diminution in fertility.

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EXPLANATION OF PLATES

PLATE 9

a. Illustrations of growth types of (left to right): 1, *C. foetida typica;* 2, F_1; 3, *C. rubra;* 4, *C. foetida rhoeadifolia* (derivative).

b. Three representatives of the population obtained by selfing the progeny of the backcross, $F_1 \times$ *foetida rhoeadifolia*.

a

b

PLATE 10

Representatives of generation of F_1 backcrossed to *rubra*, selfed.

a. Plant at left, *C rubra,* strain 1110; three plants right, derivatives of the culture 28X42, showing a close resemblance to *rubra*.

b. Left plant, *C. rubra;* center, 29Z6, a foetoid type with somatic garniture indistinguishable from *foetida* but exhibiting many *rubra* characters; right, *foetida rhoeadifolia* derivative.

c. Three plants of the culture 28X40, showing morphological gradations between the two parents: left, p. foetoid (28X40.2); center, funoid 28X40.3; right, p. foetoid (28X40.4), showing closest resemblance in branching habit to *foetida*.

a

b

c

PLATE 11

F_2 generation of *C. rubra* × *C. foetida typica.*

a. Left, amphidiploid, already past its first flowering cycle—three other cycles followed and its ultimate height approached the central plant; center, a triploid, with extra set in somatic garniture apparently *rubra* (morphology resembles *rubra* more than the amphidiploid or the plant at the right); right, a triploid with extra somatic set apparently *foetida*, and exhibiting more *foetida* characters than the other two plants (two achenes obtained).

b. 1, a sterile diploid of somatic type $C_rC_fE^1E^3$; 2, 3, and 4, triploids with extra sets resembling *foetida;* 5 and 6, fertile diploids, both with somatic garnitures of the type of plant 1.

a

b